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In the United States Patent and Trademark Office
Utility Patent Application Transmittal

Mailed: 2000, October 13

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Box Patent Application
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Please file the following enclosed patent application papers:

Applicant #1: Jeffrey A. Ledbetter
Applicant #2: Martha Hayden-Ledbetter
Title: DNA Vaccines Encoding Antigen Linked to a Domain that binds CD40

[x] Fee Transmittal Form

[x] Specification, Claims, and Abstract//Total Pages: 21

[x] Drawings//Total Pages: 7

[x] Declaration//Total Pages 3

a.Newly executed (original or copy)
b.Date Signed: 2000 Oct. 13

[x] Nucleotide and/or amino acid sequence submission
a.computer readable copy
b.paper copy, identical to computer copy

[x] Small Entity Declaration of Inventors.

[x] Return receipt postcard addressed to applicant #1.

[x] Check for $380.00 for filing fee for utility patent.

[x] Request under MPEP & 707.07(j):
The undersigned, a pro se applicant, respectfully requests that if the Examiner finds patentable subject matter disclosed in this application, but feels that Applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for applicant.

Very respectfully,

Jeffry A Ledbetter
Applicant #1 Signature

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Martha Hayden-Ledbetter
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Please type a plus sign (+) inside this box → [+]

PTO/SB/05 (4/98)
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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	
First Inventor or Application Identifier	Jeffrey Ledbetter
Title	DNA Vaccines Encoding Antigen Linked to a Domain that Binds CD40
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [Total Pages 21] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 7]
4. Oath or Declaration [Total Pages]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [X] Computer Readable Copy
   b. [X] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. [ ] Other: .....

16. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*
    [ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: _____/_____
    Prior application information: Examiner _____ Group / Art Unit: _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

[ ] *Customer Number or Bar Code Label* *(Insert Customer No. or Attach bar code label here)* or [X] *Correspondence address below*

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Signature	Jeffrey A. Ledbetter	Date	10/13/00

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

PTO/SB/17 (6/99)
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FEE TRANSMITTAL for FY 1999

Patent fees are subject to annual revision.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12. See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT ($) 380.00

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Jeffrey A. Ledbetter
Examiner Name	
Group / Art Unit	
Attorney Docket No.	

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

Deposit Account Number: _____

Deposit Account Name: _____

☐ Charge Any Additional Fee Required Under 37 CFR §§ 1.16 and 1.17

2. ☒ **Payment Enclosed:**
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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	760	201	380	Utility filing fee	380
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	760	208	380	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 380

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	Fee Paid
Total Claims	14	-20** =	0	X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

***or number previously paid, if greater; For Reissues, see below*

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	760	246	380	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	760	249	380	For each additional invention to be examined (37 CFR § 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Jeffrey A. Ledbetter	Registration No. (Attorney/Agent)		Telephone	(206) 546-0473
Signature	Jeffrey A Ledbetter		10/13/00	Date	10/13/00

PTO/SB/09 (12-97)
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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))--INDEPENDENT INVENTOR	Docket Number (Optional)

Applicant, Patentee, or Identifier: Jeffrey A. Ledbetter

Application or Patent No.: _____

Filed or Issued: _____

Title: DNA Vaccines Encoding Antigen Linked to a Domain that Binds CD40

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- [x] the specification filed herewith with title as listed above.
- [ ] the application identified above.
- [ ] the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- [x] No such person, concern, or organization exists.
- [ ] Each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

Jeffrey A. Ledbetter	Martha Hayden-Ledbetter	
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Jeffrey A Ledbetter	Martha Hayden Ledbetter	
Signature of inventor 10/13/00	Signature of inventor 10/13/00	Signature of inventor
10/13/00	10/13/00	
Date	Date	Date

Patent Application of

Jeffrey A. Ledbetter and Martha Hayden Ledbetter

For

TITLE: DNA VACCINES ENCODING ANTIGEN LINKED TO A DOMAIN THAT BINDS CD40

CROSS REFERENCE TO RELATED APPLICATIONS

This application is entitled to the benefit of Provisional Patent Application Ser. # 60/159,690, filed 1999 October 14.

BACKGROUND – FIELD OF INVENTION:

This invention relates to DNA vaccines, specifically to improved DNA vaccines that induce strong antigen-specific humoral and cellular immune responses.

BACKGROUND- DESCRIPTION OF PRIOR ART

DNA immunization, the inoculation of plasmid DNA encoding a microbial or tumor antigen, is a recent addition to vaccine technology (Donnelly J.J. et al, Ann. Rev. Immunol. 15: 617-648, 1997; Letvin N. L., Science 280: 1875-1879, 1998). Both cellular and humoral immune responses occur after DNA vaccination, and protective immunity against microbial challenge is sometimes induced in experimental animals (Ulmer J.B. et al, Vaccine 12: 1541-1544, 1994; Yokoyama M. et al, J. Virol. 69: 2684-2688, 1995; Xiang Z.Q. et al, Virology 199: 132-140, 1994; Sedegah M. et al, Proc. Natl. Acad. Sci. USA 91: 9866-9870, 1994; Montgomery D.L. et al, DNA Cell Biol. 12: 777-783, 1993). T cell responses, including CD8+ cytotoxic T lymphocyte (CTL) and CD4+ T helper cells, can be stimulated by DNA vaccination in response to antigenic peptides presented by class I and class II MHC molecules (Whitton J.L. et al, Vaccine 17: 1612-1619, 1999).

Endogenous protein synthesis allows presentation of foreign antigenic peptides by MHC class I, whereas uptake of soluble protein by APC is required for presentation of peptides by MHC class II. Both arms of the immune response can therefore be induced after DNA vaccination, but the pathways for antigen processing and presentation are distinct for peptides presented by MHC class I or MHC class II. This conclusion is derived from experiments using DNA encoding ubiquitinated protein that is rapidly targeted to intracellular degradation by proteosomes. Ubiquitinated antigen that was degraded so rapidly that intact protein could not leave the cell led to enhanced production of CTL *in vivo*, but completely eliminated antibody production (Rodriguez F. et al, J. Virol. 71: 8497-8503, 1997; Wu Y. and Kipps T.J., J. Immunol. 159: 6037-6043, 1997). Thus a major limitation of DNA vaccines is their inability to induce strong and sustained humoral immune responses. Strategies for optimization of the cellular immune response to DNA vaccines that do not reduce humoral immune responses are needed.

DNA vaccines for HIV-1 have been tested in animal models and found to induce an immune response that provides protection against challenge only when the virulence of the viral isolate is low. In benign challenge models, chimpanzees were protected from live virus exposure by vaccination with plasmid DNA or by subunit antigens or peptides (Boyer J.D. et al, Nat. Med. 3:526-532, 1997; Kennedy R.C., Nat. Med. 3: 501-502, 1997). However, when highly virulent SIV was tested in rhesus macaques, DNA vaccination was not protective and could only achieve a reduction in virus load even when multiple doses of DNA were inoculated through multiple routes (Lu S. et al, J. Virol. 70: 3978-3991, 1996). Therefore, enhancing the immune response to DNA immunization is an important goal of current AIDS vaccine research. Enhancing the immune response to other DNA vaccines is also desirable in order to provide protection when infected with highly virulent organisms or with a high infectious dose, and to provide long lasting protection. Enhancing the immune response to DNA vaccines encoding tumor antigens is also important for maximizing the anti-tumor response.

One strategy that has been tested is to prime with a DNA vaccine followed by boosting with protein antigen. However, this approach requires construction of multiple vaccines for the same infection or disease, and depends upon multiple injections given in a precise order. It would be desirable to induce protective immunity without needing

multiple forms of a vaccine, and without requiring alternating injections of DNA and protein.

Chemical and genetic approaches to enhance the immune response to DNA vaccines have been studied. Chemical adjuvants with some activity include monophosphoryl lipid A (Sasaki S. et al, Infect. Immun. 65: 3520-3528, 1997), saponin QS-21 (Sasaki S et al, J. Virol. 72: 4931-4939, 1998), mannan-coated liposomes (Toda S et al, Immunology 92: 111-117, 1997), and the aminopeptidase inhibitor ubenimex (Sasaki S et al, Clin. Exp. Immunol. 11: 30-36, 1998). Each of these adjuvants modestly enhanced both antibody titers and CTL activity after DNA vaccination in mice. Although the mechanism of action of chemical adjuvants is not fully elucidated, they seem to work by induction of cytokines that amplify responses, by recruitment of macrophages and other lymphoid cells at sites of DNA administration, or by facilitating entry of DNA into host cells (Sasaki S. et al, Anticancer Research 18: 3907-3916, 1998). Several genetic approaches to enhancing responses to DNA vaccines have been tested, including administration of a gene encoding a cytokine (IL2, IL12, GM-CSF, TCA3, MIP-1α) (Chow Y.-H. et al, J. Virol. 71: 169-178,1997; Hwee Lee A. et al, Vaccine 17: 473-479, 1998; Tsuji T. et al, Immunol.158: 4008-4014, 1997; Rodriguez D. et al, Gen.Virol.80: 217-223, 1999; Tsuji T. et al, Immunology 90: 1-6, 1997; Lu Y. et al, Clin. Exp. Immunol. 115: 335-341,1999) or a costimulatory adhesion receptor (CD86, CD58, CD54) (Tsuji T. et al, Eur. J. Immunol. 27: 782-787, 1997; Kim J.J. et al, J. Clin. Invest. 103: 869-877, 1999; Iwasaki A. et al, J. Immunol. 158: 4591-4601, 1997). Each of these cytokine and adhesion receptor genes increased immune responses to DNA vaccination, with some treatments enhancing CTL generation only, and some enhancing both CTL and antibody production. However, the levels of enhancement of the immune response to DNA vaccination obtained from these approaches are modest and not sustained, so it is important to find additional ways to enhance the immune response to DNA vaccines.

The CD40 receptor must be activated for an effective cellular or humoral immune response after exposure to antigen (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998). This conclusion is derived from multiple findings, including the phenotype of patients with hyper IgM (HIGM) syndrome that results from CD154

genetic defects (Aruffo A. et al, Cell 72: 291-300,1993; Fuleihan R. et al, Proc. Natl. Acad. Sci. USA 90: 2170-2173,1993; Korthauer U. et al, Nature 361: 539-541,1993), the phenotype of mice with CD40 or CD154 gene disruption (Grewal I.S. et al, Science 273: 1864-1867,1996; Kawabe T. et al, Immunity 1: 167-178,1994; Renshaw B. et al, J. Exp. Med. 180: 1889-1900,1994; Xu J. et al, Immunity 1: 423-431, 1994), and the effects of actively blocking CD40 *in vivo* using inhibitory antibodies to CD154 (Durie F.H. et al, Science 261: 1328-1330,1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163,1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). CD40 is expressed in several cell lineages, including B cells, dendritic cells, monocytes, epithelial cells, and endothelial cells. CD40 transmits signals for each of these cell types that regulates activation and differentiation (Hollenbaugh D. et al, EMBO J. 11: 4313-4321,1992; Kiener P.A. et al, J. Immunol. 155: 4917-4925,1995; Cella M. et al, J. Exp. Med. 184: 747-752,1996; Galy A.H., and Spits H., J. Immunol. 152: 775-782,1992; Clark E.A., and Ledbetter J.A., Proc. Natl. Acad. Sci. USA 83: 4494-4498, 1986). CD40 is activated by crosslinking during cell to cell contact with cells expressing CD40 ligand (CD154), primarily T cells. While soluble forms of CD154 can stimulate CD40, no attempts have been made to use or modify soluble CD154 to promote immune responses to antigens.

CD40 signals to B cells are required for isotype switching and affinity maturation through somatic mutation (Rousset F. et al, J. Exp. Med. 173: 705-710, 1991). In the absence of CD40 signals, germinal centers, the specialized sites of B cell maturation, are not formed, and B cells are unable to differentiate into IgG producing plasma cells (Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994). Patients with HIGM syndrome are not able to form germinal centers or produce IgG antibodies after antigen challenge, and the same phenotype is seen in knockout mice where CD40 or CD154 is not expressed. The CD40 signal has been shown *in vitro* to promote survival of surface Ig-activated B cells, and to interact with signals from cytokines to induce immunoglobulin isotype switching to IgG, IgA, and IgE production (Holder M.J. et al, Eur. J. Immunol 23: 2368-2371,1993; Jabara H.H. et al, J. Exp. Med. 177: 925-935,1990; Grabstein K.H. et al, J. Immunol. 150: 3141-3147, 1993). In addition, HIGM syndrome patients and CD154 knockout mice have impaired lymphocyte proliferation in response to diphtheria toxoid,

tetanus, and *Candida*, showing that the CD40 signal is required for T cell priming to protein antigens (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998; Toes R.E.M. et al, Sem. Immun. 10: 443-448,1998; Grewal I.S. et al, Nature 378: 617-620,1995; Ameratunga R. et al, J. Pediatr. 131: 147-150,1997; Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999). Expression of CD154 *in vivo* to enhance immune responses utilized only the cell surface form of the molecule and resulted in significant toxicity in experimental animals, including induction of lethal autoimmune disease and T cell malignancies (Roskrow M.A et al, Leukemia Research 23: 549-557, 1999; Brown M.P. et al, Nature Medicine 4: 1253-1260, 1998).

In neonates, insufficient stimulation of CD40 due to low levels of expression of CD154 by activated T cells has been identified as a factor in the inability of infants to produce IgG antibodies towards bacterial antigens (Nonoyama S. et al, J. Clin. Invest. 95: 66-75, 1995; Fuleihan R. et al, Eur. J. Immunol. 24: 1925-1928, 1994; Brugnoni D. et al, Eur. J. Immunol. 24: 1919-1924, 1994). This suggests that CD40 signals are not ubiquitous and that highly restricted expression of CD154 may limit the extent of CD40 signaling and thus the magnitude and quality of an immune response. Direct evidence in support of this idea comes from a recent study where a modest increase (1.1-2 fold) in expression of cell surface CD154 in the thymus of mice resulted in a > 10 fold increase in the antigen-specific antibody response (Prez-Melgosa M. et al, J. Immunol. 163: 1123-1127, 1999). Some evidence suggests that CD40 stimulation may be deficient in HIV-1 infected individuals, since HIV gp120 suppressed the expression of CD154 by activated T cells *in vitro*, and production of IL12 is defective in HIV-1 positive individuals (Chirmule N. et al, J. Immunol. 155: 917-924, 1995; Taoufik Y. et al, Blood 89: 2842-2848, 1997; Yoo J. et al, J. Immunol. 157: 1313-1320, 1996; Ito M. et al, AIDS Res. Hum. Retroviruses 14: 845-849, 1998; Benyoucef S. et al, J. Med. Virol. 55: 209-214, 1998). In addition, CD40 stimulation of dendritic cells infected with HIV-1 was found to suppress virus replication, suggesting that transmission of HIV-1 from infected dendritic cells during antigen presentation could be blocked by CD40 signals (McDyer J.F. et al, J. Immunol. 162: 3711-3717, 1999). However, a method for stimulation of CD40 on cells actively presenting antigen to T cells while avoiding toxicity from unregulated CD40 stimulation is needed.

CD40 signals to dendritic cells or B cells causes their differentiation from an antigen uptake function to an antigen processing and presentation function (Sallusto D. et al, J. Exp. Med. 182: 389-400, 1995; Cella M. et al, J. Exp. Med. 184: 747-752, 1996; Faassen A.E. et al, Eur. J. Immunol. 25: 3249-3255, 1995). This shift is accompanied by reduction of the MHC class II intracellular compartment, increased expression of MHC class II on the cell surface, secretion of the Th1 regulatory cytokine IL12 and increased expression of CD86 and CD80. After CD40 activation, dendritic cells and B cells are able to more efficiently present antigen and give a critical costimulatory signal through CD28. The production of IL12 leads to enhanced secretion of IFNγ by T cells and suppression of Th2 cytokine production. The CD40 signal is therefore an important mediator of Th1 cellular immunity and CTL induction. However, selective stimulation of CD40 during antigen presentation is needed to enhance immune responses to vaccination.

In addition to B cells and dendritic cells, CD40 is functionally active on other APC's such as monocytes, where CD40 signals prevent cell death from apoptosis and induce expression of adhesion molecules and production of inflammatory cytokines TNFα and IL8 (Kiener P.A. et al, J. Immunol. 155: 4917-4925, 1995). CD40 has also been reported to be expressed and functionally active on thymic epithelial cells (Galy A.H., and Spits H., J. Immunol. 152: 775-782, 1992) and on many kinds of tumor cells, including carcinomas, melanomas, and lymphomas (Ledbetter J.A. et al, In Leucocyte Typing III: White Cell Differentiation Antigens p. 432-435, 1987; Oxford University Press, Oxford, U.K.; Paulie S. et al, Cancer Immunol. Immunother. 20: 23-28, 1985). In contrast to most normal cells where the CD40 signal enhances survival, in many malignant cells CD40 actively promotes death by apoptosis. Therefore CD40 is functionally active in all cell types that express the receptor, and CD40 signals are central to fundamental processes of survival and differentiation. Because of the widespread expression of functional CD40, localized stimulation of CD40 positive cells that present specific antigen to T cells is desirable so that only APC involved in the specific immune response are activated.

Studies in CD154 knockout mice have confirmed the importance of CD40 activation for the antigen specific priming of T cells. CD154 deficient mice have an

enhanced susceptibility to *Leishmania major* and *Toxoplasma gondii* infection, consistent with a central role for CD40 in cellular immunity (Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999; Campbell K.A. et al, Immunity 4: 283-289, 1996). CTL generation after viral infection in CD154 deficient mice is markedly blunted, and induction of experimental allergic encephalomyelitis (EAE) in response to myelin basic protein does not occur (Grewal I.S. et al, Science 273: 1864-1867, 1996; Grewal I.S. et al, 378: 617-620, 1995). The defect in T cell priming in these models appears to be due to an inability of APC to provide costimulatory signals to T cells (Grewal I.S. et al, Science 273: 1864-1867, 1996; Yang Y. and Wilson J.M., Science 273: 1862-1867, 1996).

Inhibition of CD40 *in vivo* has been studied in mice using a mAb, MR1, that binds and blocks the CD40 ligand, CD154 (Durie F.H. et al, Science 261: 1328-1330, 1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). These experiments demonstrated that anti-CD154 prevents the induction of autoimmune diseases, including EAE after immunization with myelin basic protein, oophritis after immunization with zona pelucida antigen (ZP3), and spontaneous disease in lupus prone mice (Griggs N.D. et al, J. Exp. Med. 183: 801-807, 1996; Daikh D.I. et al, J. Immunol. 159: 3104-3108, 1997). Anti-CD154 was also effective in preventing both chronic and acute graft versus host (GVH) disease and in preventing rejection of heart allografts after transplantation (Larsen C.P. et al, Nature 381: 434-438, 1996). Thus, CD40 signals are required for T cell responses to antigen, and restriction of the CD40 signal with specific inhibitors is an effective method of limiting T cell priming during an immune response.

The CD40 receptor is therefore a proven target for regulation of antigen specific immunity. While biological inhibitors of CD40 have been studied extensively in mice and in nonhuman primates, there is a need for localized stimulation of CD40 on cells that present antigens to T cells in order to improve the effectiveness of vaccines.

Gp160, the product of the HIV-1 env gene, is cleaved in the Golgi complex into gp120 and gp41 proteins that remain associated through noncovalent interactions. Most

neutralizing epitopes of the virus are located on gp120 and gp41, and are expressed by the intact env complex that has been shown to be a trimer (Kwong P.D. et al, Nature 393: 648-659, 1998). Monomeric gp120 can be released from the complex and expose immunodominant epitopes that are non-neutralizing and are located on the internal face of gp120 in the intact trimeric complex (Wyatt R. et al, Nature 393: 705-711, 1998; Broder C.C. et al, PNAS USA 91: 11699-11703, 1994). Thus, stabilization of the env complex is needed for an HIV-1 vaccine in order to preserve conformational epitopes important for neutralization and to mask immunodominant epitopes that are not relevant for neutralization of the env complex.

One attempt to produce a stable, properly folded gp120-gp41 complex was made by altering the cleavage site in gp160 between the gp120 and gp41 domains (Earl P.L. et al, J. Virol. 68: 3015-3026, 1994). By introducing a stop codon before the transmembrane domain of gp41, a soluble molecule composed of gp120 and the extracellular domain of gp41 was produced as a complex that folds properly to bind the CD4 receptor and to express some conformational epitopes. However, this molecule formed dimers and multimers rather than the stable trimers that comprise the native structure of the envelope glycoprotein as revealed in the crystal structure of the gp120 complex.

Three major sites of gp120 have been identified that are involved in cross-neutralization of diverse viral strains (Wyatt R. et al, Nature 393: 705-711, 1998). The V3 domain was found to express linear and conformational epitopes that can be recognized by antibodies that neutralize HIV-1. Although the V3 domain is a variable region, it contains a central portion shared by many HIV-1 isolates, particularly those found in the United States and Europe. The central portion has been called the principle neutralization epitope and is formed from a linear epitope of the amino acid sequence GPGRAF (Broliden P.A. et al, Proc. Natl. Acad. Sci. USA 89: 461-465, 1992; Broliden P.A. et al, Immunol. 73: 371-376, 1991; Javaherian K. et al, Science 250: 1590-1593, 1990; Javaherian K. et al, Proc. Natl. Acad. Sci. USA 86: 6768-6772, 1989). Conformational epitopes of the V3 loop have also been identified that can be recognized by antibodies that are more broadly neutralizing.

The CD4 binding domain of gp120 is another neutralization site for antibodies directed to HIV-1 env. This domain is a nonlinear, conformational site that depends upon proper folding of gp120 (Kang C.-Y. et al, Proc. Natl. Acad. Sci. USA : 6171-6175, 1991; Lasky L.A. et al, Cell 50: 975-985, 1987). Antibodies can recognize distinct portions of the CD4 binding domain, and may have either type-specific or cross-neutralization properties (Pinter A. et al, AIDS Res. Hum. Retro. 9: 985-996, 1993). Although monomeric gp120 can retain CD4 binding function, a stable trimeric structure of gp120 is thought to be important for masking immunodominant epitopes that are expressed on the internal face of the intact complex (Wyatt R. et al, Nature 393: 705-711, 1998). A third domain of gp120 involved in virus neutralization is exposed upon binding to CD4, and functions to bind the chemokine coreceptor to allow virus entry into the cell (Rizzuto C.D. et al, Science 280: 1949-1953, 1998). Thus a stable trimer of HIV-1 env is needed to present the major cross-neutralization epitopes and to prevent exposure of internal, immunodominant epitopes that do not induce neutralizing antibodies.

CD154 is a TNF-related, type II membrane protein that forms stable trimers (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). Soluble fusion proteins of human CD154 have been expressed using murine CD8 at the amino terminal side of the CD154 molecule (Hollenbaugh D. et al, EMBO J. 11: 4313-4321, 1992). Single chain Fv (scFv) molecules have also been constructed using heavy and light chain variable regions cloned from the G28-5 hybridoma that produces antibody specific for human CD40 (Ledbetter J.A. et al, Crit. Rev. Immunol.17: 427-435, 1997). Both CD154 and G28-5 scFv fusion proteins retain functional activity as soluble molecules *in vitro*. However, no use of these molecules to improve the effectiveness of vaccines has been found.

SUMMARY

For vaccines to be effective, they must induce both humoral and cellular immune responses. This invention describes improved vaccines that target antigens to cell surface receptors. DNA vaccines are a recent addition to immunization technology. However, further optimization of DNA vaccines is needed to induce long-lasting

protection against tumor antigens, virulent HIV-1 isolates, and other pathogenic microorganisms. Receptor activation and targeting improves the ability of DNA vaccines to generate strong cellular immunity and high titers of neutralizing antibodies. CD40 is a preferred receptor for targeting and activation. DNA vaccines encoding CD40 ligand (CD154) or a single chain Fv (scFv) specific for CD40, fused with DNA encoding portions of the HIV-1 env protein are preferred embodiments of the invention. A molecule comprising the extracellular domain of HIV-1env gp160 or env gp120 linked to the extracellular domain of CD154 is a stable trimer that improves immune recognition of HIV-1 env cross-neutralization epitopes. After DNA vaccination, the expression of the fusion protein *in vivo* results in both activation of the CD40 receptor and direction of HIV-1 env antigens into the endocytic pathway of CD40 positive antigen presenting cells (APC). Internalization of env antigens after binding the CD40 receptor enhances presentation of peptides by MHC molecules. Activation of the CD40 receptor promotes B cell and APC maturation leading to effective antibody production and generation of CD4+ helper T cell and CD8+ CTL activity. The combination of CD40 activation, stabilization of the HIV-1 gp160 or gp120 env trimer, and enhanced presentation of antigenic peptides by MHC molecules thus improves immune responses to HIV-1 antigens. Protein molecules of the invention can be injected directly into mammals or encoded by DNA vaccines.

DRAWINGS

Figure 1.

Schematic representation of fusion proteins that target antigen to cell surface receptors expressed by antigen presenting cells.

A. A fusion protein expressed from a cDNA construct that encodes an antigen domain attached with a linker to a receptor targeting domain. The antigen domain may be attached to the amino terminus of the receptor targeting domain as shown, or may be attached to the carboxy terminus of the receptor targeting domain.

B. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of the CD154 extracellular domain.

C. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of a single chain Fv specific for CD40.

D. A fusion protein expressed from a cDNA construct as in C, except that the scFv that binds CD40 is oriented with the light chain variable region (V_L) attached to the carboxy-terminus of the heavy chain variable region (V_H).

E. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a camelid variable region (V_{HH}) that binds CD40.

F. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a peptide that binds CD40.

Figure 2.

A. Sequence of two cDNAs encoding HIV gp120-V3 loop/CD154 long form extracellular domain fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV V3 loop from gp120 with a (ProAspPro) linker (SEQUENCE ID NO.: 17 [DNA] OR SEQUENCE ID NO.: 25 [FUSION PROTEIN]) or a ($Gly_4Ser)_3$ linker (SEQ. ID NO.: 16 [DNA] OR SEQ. ID NO.:24 [FUSION PROTEIN]) fused to the CD154 extracellular domain encoded between amino acids 48 (Arg)-261(Leu), with an additional (Glu) residue at the carboxyl end of the protein, not present in wild type CD154. The sequence of the fusion protein is indicated using the three-letter amino acid code convention, above each codon of the open reading frame. Relevant restriction sites are indicated on the drawing and the nucleotides encoding sites at domain fusion junctions are displayed in boldface type, while the first codon of each fused domain is indicated in underlined, italicized type. The protein domains are labeled above the relevant position in the sequence. The nucleotide number is indicated in the left margin with a designation for the PDP linker form or the G4S linker form.

B. Sequence of two cDNAs encoding HIV V3 loop-CD154 short form extracellular domain fusion proteins.

The two HIV V3 loop constructs with alternate linkers, either (ProAspPro) (SEQUENCE ID NO.:19 [DNA] OR SEQUENCE ID NO.: 27 [FUSION PROTEIN]) or ($Gly_4Ser)_3$ (SEQUENCE ID NO.: 18 [DNA] OR SEQUENCE ID NO.: 26 [FUSION PROTEIN])

were also fused to the short form of the CD154 extracellular domain encoded from amino acids 108 (Glu)-261 (Leu) plus an extra glutamic acid residue at the carboxy terminus, not encoded by wild type CD154. All sequences are labeled as described for Figure 2A.

Figure 3.

A. Sequence of two HIV gp120env-CD154 long form extracellular domain cDNA and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 13 [DNA] OR SEQ. ID NO.: 21 [FUSION PROTEIN]) or a $(Gly_4Ser)_3$ linker (SEQ. ID NO.: 12 [DNA] OR SEQ. ID NO.: 20 [FUSION PROTEIN]) fused to the CD154 extracellular domain (Long Form) encoded between amino acids 48 (Arg)-261(Leu) + (Glu). All sequences are labeled as described for Figure 2A.

B. Sequence of two HIV gp120env-CD154 short form extracellular domain cDNAs and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 15 [DNA] or SEQ. ID NO.: 23 [fusion protein]) or a (Gly4Ser)3 linker (SEQ. ID NO.: 14 [DNA] or SEQ. ID NO.: 22 [fusion protein]) fused to the short form of the CD154 extracellular domain encoded between amino acids 108 (Glu)-261 (Leu) + (Glu).. All sequences are labeled as described for Figure 2A.

DESCRIPTION

This invention relates to improved vaccines comprising one or more antigens attached to a domain that targets at least one cell surface receptor. The vaccine may be delivered either as a protein, as a DNA plasmid, or by a viral vector. The expression of the DNA after injection of the plasmid or viral vector *in vivo* results in the secretion of the antigen(s) attached to a targeting domain, directing the antigen(s) to a cell surface receptor. Receptor-mediated internalization of the antigen into the endocytic compartment of cells that express the receptor enhances the presentation of antigenic peptides by MHC class II molecules that circulate through this compartment.

Presentation of antigenic peptides by MHC class I molecules is mediated by the cells expressing the DNA vaccine, and is enhanced in cells that internalize the antigen-targeting domain fusion protein by movement of the fusion protein from the endocytic compartment into the cytoplasm. The activation of antigen-specific CD4+ T cells and CD8+ T cells is increased, resulting in better humoral and cellular immune responses.

The preferred receptor(s) chosen for antigen targeting are those expressed by antigen presenting cells (APC), such as dendritic cells. Desirable receptors for targeting include but are not limited to CD80, CD86, CD83, CD40, CD32, CD64, Flt3, Dec 205, and ICOS ligand. The CD40 receptor is a preferred receptor for antigen targeting, since signals from CD40 regulate activation and differentiation of APC. Fusion proteins of antigen and CD154 (CD40 ligand) combine the functions of antigen targeting and activation of APC by simultaneous delivery of CD40 signals.

The preferred antigen(s) for receptor targeting are HIV-1 and HIV-2 viral antigens, since vaccines have not been effective in protecting against virulent viral isolates. Attachment of HIV-1 gp160 or gp120 extracellular domain to CD154 extracellular domain stabilizes the trimeric structure of HIV-1 env. However, the invention is not limited to HIV env antigens, since improved immune responses to vaccines are needed to provide long-lasting protection against infection with high doses of pathogenic microorganisms or against tumors.

Thus the structure of the invention's main embodiment is a DNA plasmid encoding the extracellular domain of HIV-1 env gp160 attached to the CD154 extracellular domain.

The fusion protein expressed from this DNA plasmid a) stabilizes the trimeric structure of HIV-1 env, b) directs the HIV-1 antigen into the MHC class II compartment of CD40 positive cells, and c) selectively activates the CD40 receptor to increase APC functional activity.

The main embodiment of the invention encodes a stable trimer that expresses the major cross-neutralization epitopes of HIV-1 env while masking the internal env

epitopes that are not involved in virus neutralization. Antigenic peptides of HIV env are presented by MHC class I molecules by cells that express the DNA, while antigenic peptides of HIV env are presented by MHC class II molecules in CD40 positive cells that internalize the trimeric antigen-CD154 fusion protein. Activation of the CD40 receptor on cells bound by the antigen-CD154 fusion protein increases the specific immune response due to increased production of IL12 and increased expression of costimulatory molecules CD80 and CD86.

OPERATION

An improved DNA vaccine for AIDS comprising the extracellular domain of HIV-1 gp160, HIV-1 gp120, or a subdomain of these antigens fused to the extracellular domain of CD154 is described. Alternative embodiments of the invention use a smaller portion of the CD154 molecule composed of an 18 kDa subunit from Glu-108 to Leu-261 (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). The extracellular domain of gp160 can also be shortened by removing the gp41 domain, removing the V1 and V2 domains, or mutating the glycosylation sites without damaging the conformational structure of the HIV-1 envelope (Kwong P.D. et al, Nature 393: 648-659, 1998). These changes could further improve the activity of the vaccine, since the V1 and V2 loops, and the carbohydrate structures are thought to be exposed, clade specific epitopes that prevent or dilute the immune response to important cross-neutralization epitopes for diverse clades of HIV-1. Linkers between gp160 and CD154 can also be used. Thus, alternative embodiments of the invention minimize the CD154 domain, remove gp41, V1, V2, or glycosylation sites of gp160. This invention also envisions DNA vaccines comprising other HIV-1 antigens and antigens from alternative isolates of HIV-1, fused to the extracellular domain of CD154.

Delivery of antigen(s) to the CD40 receptor may use anti-CD40 scFv instead of CD154. Single antibody variable regions (V_{HH}) or peptides that bind CD40 are also included in the scope of the invention.

Antigen targeting to receptors is not limited to the CD40 receptor. Alternative receptors preferred for targeting include CD80, CD86, Dec205, ICOS ligand, Flt 3, Fc

receptors, and CD83. All cell surface receptors are envisioned by this invention. Receptors may be targeted by ligands, scFv molecules, single variable regions or peptides. Additional methods of attachment of antigen(s) to receptor targeting domains are envisioned, including chemical linkages of subunits, disulfide bonds, or noncovalent attachments such as leucine zipper motifs and the like. The invention contemplates injection of protein, injection of DNA plasmids, or viral vectors encoding the molecules comprising one or more antigens linked to a receptor-binding domain.

Antigens targeted to cell surface receptors are not limited to HIV gp160 antigens. Other antigens, including tumor antigens, parasite antigens, bacterial antigens, and viral antigens are included in the scope of the invention.

The invention also envisions delivery of antigens to cell surface receptors in order to induce antigen-specific tolerance or nonresponsiveness. For this application, an autoantigen would be chosen and the vaccine would be used to treat autoimmune disease.

The invention also envisions antigen(s) that are natural components of the body, such as tumor-associated antigens, where an immune response to the antigen(s) breaks tolerance to the antigen, resulting in a change in immune homeostasis.

The following examples describe particular embodiments of the invention but are not meant to limit its scope.

EXAMPLE 1

A preferred embodiment of the DNA vaccine includes an amino-terminal secretory signal peptide sequence upstream and adjacent to a cDNA sequence cassette encoding the desired antigen. This molecule is then fused to the extracellular domain of CD154 or to a portion of the extracellular domain of CD154 which retains the ability to bind CD40, or to an scFv targeted to CD40, to create a fusion protein expression cassette that targets the antigen to the antigen presenting cell through the CD40 receptor as diagrammed in Figure 1. The expression cassette is inserted into an appropriate mammalian expression vector or virus to achieve high level expression of the fusion protein either *in vitro* or *in vivo*.

The leader peptide is encoded on complementary oligonucleotides with a single-stranded HindIII cohesive end at the 5' terminus, and a BglII cohesive end at the 3' terminus. The sense oligonucleotide is designated SEQUENCE ID NO: 1 or HBLPS and the sequence is as follows:

5'**agct**tgccgccatgctgtatacctctcagctgttaggactacttctgttttggatctcggcttcga-3'.

The antisense oligonucleotide is designated SEQUENCE ID NO: 2 or HBLPAS and the sequence is as follows:

5'**gatc**tcgaagcccgagatccaaaacagaagtagtcctaacagctgagaggtatacagcatggcggca-3'. The two molecules anneal to one another except at the overhanging nucleotides indicated in boldface type. Alternative embodiments could include other secretory signal peptides or localization sequences.

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). Two different fusion junctions were designed which resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu) + (Glu),, and a long or complete form (form L2) including amino acids 48 (Arg) - 261 (Leu) + (Glu), of the extracellular domain of CD154. The sense primer which fuses the extracellular domain to the targeted antigen includes a BamHI site for cloning that introduces the peptide sequence PDP or (ProAspPro) at the fusion junction and can also encode a linker peptide such as $(Gly_4Ser)_3$ to separate the antigen from the extracellular domain. The oligonucleotide primers used in amplifying the short form (S4) of the CD154 extracellular domain encoding amino acids 108 (Glu)-261 (Leu) + (Glu) are as follows:

The sense primer is designated SEQUENCE ID NO: 3 or CD154BAM108 and encodes a 34 mer with the following sequence : 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO: 4 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

The oligonucleotide primers used in amplifying the long form (L2) of the CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), are as follows: The sense primer is identified as SEQUENCE ID NO: 5 or CD154 BAM48 and encodes a 35 mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-

3', while the antisense primer is also SEQUENCE ID NO: 4 or CD154XBA encoding the 44 mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

A variety of different antigens can be encoded on cDNA cassettes to be inserted between the leader peptide cassette and the CD40 targeted domain (such as a truncated or complete CD154 extracellular domain or a CD40 specific scFv). In a preferred embodiment of the invention, the cDNA antigen encoded by the vaccine is the HIV-1 gp 120 or a fragment of this antigen, such as the V3 loop. The primer sets used to amplify the complete gp120 domain include the sense primer SEQUENCE ID NO: 6 or GP120Bgl2f 5'-gga tat tga tga gat cta gtg cta cag-3' and one of two antisense primers encoding different linkers. Either the antisense primer encoding the ProAspPro linker, identified as SEQUENCE ID NO: 7 or GP120PDPr 5'-gaa cac agc tcc tat tgg atc cgg tct ttt ttc tct ttg cac-3' or the antisense primer encoding the $(Gly_4Ser)_3$ linker, identified as SEQUENCE ID NO: 8 or GP120G4Sr 5'-cct gca tgg atc cga tcc gcc acc tcc aga acc tcc acc tcc tga acc gcc tcc ccc tct ttt ttc tct ttg cac tgt tct tct ctt tgc-3' were used to amplify the gp120 domain with the desired linker attached. PV75Kgp160(89.6) DNA was used as template in PCR reactions. Alternatively, other isolates or sequence variants of gp120 or gp160 are available and can be substituted to create novel fusion cassettes. PCR amplification reactions were performed using cloned plasmid DNA as template (approximately 45 ng), 3 mM $MgCl_2$, 0,3 MM dNTPs, 1/10 volume 10X reaction buffer supplied by the manufacturer, 10 pmol sense primer, 10 pmol antisense primer, and 2.5 units TAQ polymerase (Takara Pharmaceuticals) in a total reaction volume of 50 µl. The amplification profile included an initial 4 minute 94°C denaturation, followed by a 30 cycle program of 50°C annealing for 30 seconds, 72°C extension for 30 seconds, and 94°C denaturation for 30 seconds. PCR fragments were purified by ethanol precipitation, resuspended in 30 µl ddH_2O and 10 µl was digested with BglII (Roche) restriction endonuclease in a 20 µl reaction volume at 37°C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN, San Diego, CA), and ligated along with the annealed leader peptide oligonucleotides to HindIII-BamHI digested expression vector already containing the CD154 extracellular domain as a BamHI-XbaI fragment. Recombinant clones were screened for the correct orientation and presence of inserts, and the resulting positive clones were verified by DNA sequencing using an ABI 310 sequence analyzer and the ABI Prism Dye Terminator Reaction Chemistry. The final fusion cassette encodes the

synthetic leader peptide fused to the HIV gp120 domain with either a (ProAspPro) linker or a $(Gly_4Ser)_3$ linker, and then to the CD154 extracellular domain long (Figure 3A) or short (Figure 3B) form to create the embodiments of example 1.

EXAMPLE 2

In an alternative preferred embodiment, the V1 and V2 domains of gp120 are removed and only the V3 loop domain from HIV gp 120 is encoded on a BglII-BamHI fragment and fused to the signal peptide and the CD154 extracellular domain to create the vaccine, as illustrated in Figure 2A and B. This antigen domain is separated from the CD154 short (Figure 2B) or long extracellular domain (Figure 2A) by a peptide linker encoding the amino acids (ProAspPro), or a longer peptide linker encoding the amino acids $(Gly_4Ser)_3$.

The V3 loop was PCR amplified from pV75 (gp 89.6), a plasmid containing HIV gp120 from isolate LAV, using the following primer set:

The antisense primer encoding a ProAspPro linker is SEQUENCE ID NO: 9 or V3PDPr

5'-gtt att cca tgg atc cgg act aat ctt aca atg tgc ttg-3'

The sense primer fusing the antigen to the signal peptide is SEQUENCE ID NO: 10 or V3Bgl2f

5'-gta cag cta aat aga tct gta gta att aat tg-3'

The antisense primer encoding a $(Gly_4Ser)_3$ linker is SEQUENCE ID NO: 11 or V3G4Sr

5'-ggt gca tgg atc cga acc tcc acc gcc aga tcc acc gcc tcc tga ggc acc gcc acc act aat gtt aca atg tgc ttg ttg tct tat atc tcc-3'.

Amplification, digestion, purification, and ligation conditions were identical to those described above for the full-length gp120 domain. The final fusion cassettes encode the HIV gp120-V3 loop with either a (ProAspPro) linker or a $(Gly_4Ser)_3$ linker fused to either the CD154 extracellular domain as diagrammed in Figure 2A for the long form, and Figure 2B for the short form of the CD40 binding domain.

Other antigens and linkers can be substituted to create alternative vaccines by construction of the appropriate cDNA cassettes encoding the desired domains and attaching them to the CD154 extracellular domain. Because of the high degree of sequence variation among HIV isolates, alternative sequences might be incorporated as needed to target particular clades. Other viral antigens such as HIV tat or their

subdomains can be substituted for the HIV domains described here. Similarly, an alternate APC targeted domain can be substituted for the CD40 binding domain, such as a domain which binds to CD80 or CD86, or to ICOS ligand, or to one of several other cell surface receptors expressed on antigen presenting cells. Surface receptors that internalize readily are preferred over receptors that contain multiple transmembrane domains and do not internalize readily such as G-protein coupled chemokine receptors.

CLAIMS: We claim:

1. A vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
2. A vaccine of claim 1 where said receptor is CD40.
3. A vaccine of claim 1 where said domain is CD154 or a portion of CD154.
4. A vaccine of claim 1 where said domain is a single chain Fv that binds CD40.
5. A vaccine of claim 1 where said domain binds to one or more receptors selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
6. A vaccine of claim 1 where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
7. A vaccine of claim 1 where said antigen is a tumor antigen or a microbial antigen.
8. A DNA expression plasmid encoding a vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
9. A DNA expression plasmid of claim 8 encoding a vaccine where said receptor is CD40.
10. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is CD154 or a portion of CD154.
11. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is a single chain Fv that binds CD40.
12. A DNA expression plasmid of claim 8 encoding a vaccine where said domain binds to one or more antigens selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
13. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
14. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is a tumor antigen or a microbial antigen.

ABSTRACT

Vaccines that target one or more antigens to a cell surface receptor improve the antigen-specific humoral and cellular immune response. Antigen(s) linked to a domain that binds to a cell surface receptor are internalized, carrying antigen(s) into an intracellular compartment where the antigen(s) are digested into peptides and loaded onto MHC molecules. T cells specific for the peptide antigens are activated, leading to an enhanced immune response. The vaccine may comprise antigen(s) linked to a domain that binds at least one receptor or a DNA plasmid encoding antigen(s) linked to a domain that binds at least one receptor. A preferred embodiment of the invention targets HIV-1 env antigen to the CD40 receptor, resulting in delivery of antigen to CD40 positive cells, and selective activation of the CD40 receptor on cells presenting HIV-1 env antigens to T cells.

Figure 1.
Fusion Proteins that Target Antigen to APC

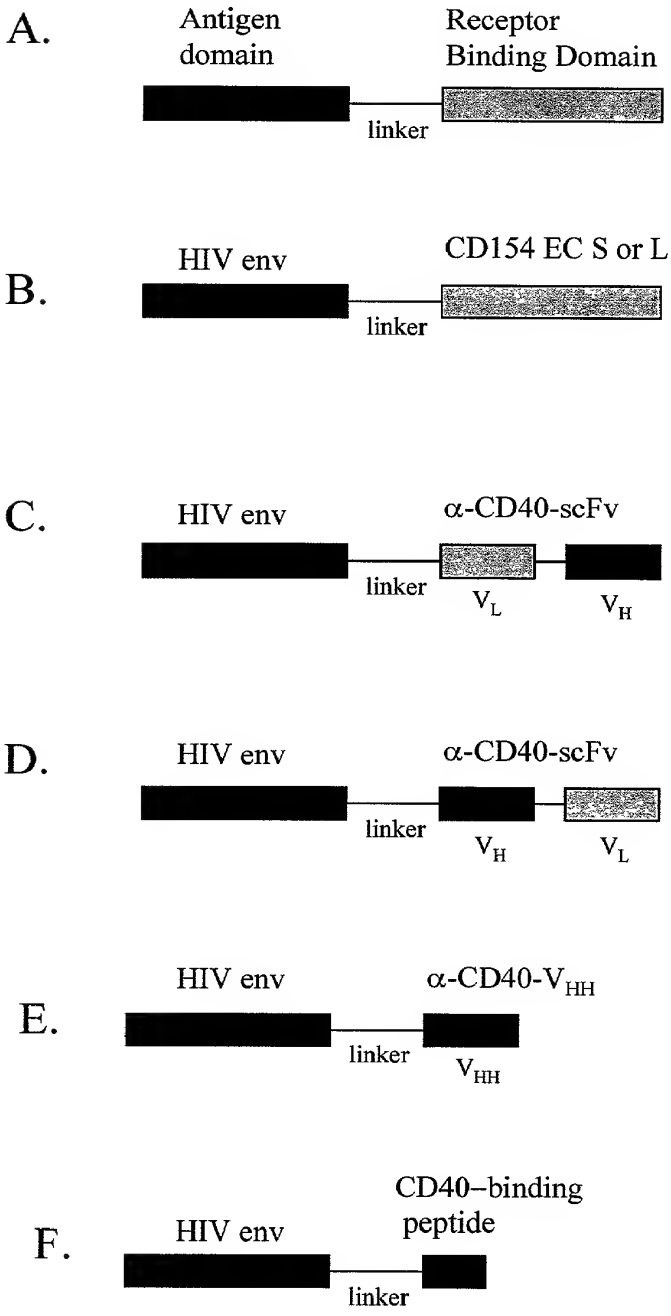

Figure 2A.

Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-CD154 LONG form extracellular domain fusion proteins.

```
        HindIII
        ~~~~~~~~~        Signal Peptide
                         Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
   1    AAG CTT GCC GCC  ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                     BglII
                                     ~~~~~~~~~ HIVgp120-V3 loop
        Leu Phe Trp Ile Ser Ala Ser Arg Ser Val Val Ile Asn Cys Thr
  46    CTG TTT TGG ATC TCG GCT TCG AGA TCT GTA GTA ATT AAT TGT ACA
        Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly
  91    AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA TCT ATA GGA CCA GGG
        Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln
 136    AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA GGA GAT ATA AGA CAA
        Ala His Cys Asn Ile Ser
 181    GCA CAT TGT AAC ATT AGT
        ProAspPro Linker
           BamHI
           ~~~~~~~~~
        Pro Asp Pro
 199    CCG GAT CCA

        OR (Gly4Ser)3 Linker                                       BamHI
                                                                   ~~~~~~~~~
        Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
 199    GGT GGC GGT GGC TCA GGA GGC GGT GGA TCT GGC GGT GGA GGT TCG GAT CCA

            CD154 LONG extracellular domain
208PDP      Arg Arg Leu Asp Lys Ile Glu
250GS       AGA AGG TTG GAC AAG ATA GAA
229PDP      Asp Glu Arg Asn Leu His Glu Asp Phe Val Phe Met Lys Thr Ile
271GS       GAT GAA AGG AAT CTT CAT GAA GAT TTT GTA TTC ATG AAA ACG ATA
274PDP      Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys
316GS       CAG AGA TGC AAC ACA GGA GAA AGA TCC TTA TCC TTA CTG AAC TGT
319PDP      Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys Asp Ile Met
361GS       GAG GAG ATT AAA AGC CAG TTT GAA GGC TTT GTG AAG GAT ATA ATG
364PDP      Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln
406GS       TTA AAC AAA GAG GAG ACG AAG AAA GAA AAC AGC TTT GAA ATG CAA
409PDP      Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu
451GS       AAA GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG
454PDP      Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
496GS       GCC AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA
499PDP      Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys
541GS       TAC TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA
544PDP      Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
586GS       CAG CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA
589PDP      Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe
631GS       GTC ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT
634PDP      Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
676GS       ATA GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC
679PDP      Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly
721GS       TTA CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG
724PDP      Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
766GS       CAA CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT
769PDP      Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His
811GS       GCT TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT
814PDP      Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** ***
856GS       GGC ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA
            XbaI
859PDP      ~~~~~~~
901GS       TCT AGA
```

Figure 2B.

Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-CD154 SHORT form extracellular domain fusion proteins.

```
        HindIII
        ~~~~~~~~        Signal Peptide
                        Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
   1    AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                BglII HIVgp120-V3 loop
                                ~~~~~~~~
        Leu Phe Trp Ile Ser Ala Ser Arg Ser Val Val Ile Asn Cys Thr
  46    CTG TTT TGG ATC TCG GCT TCG AGA TCT GTA GTA ATT AAT TGT ACA
        Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly
  91    AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA TCT ATA GGA CCA GGG
        Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln
 136    AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA GGA GAT ATA AGA CAA
        Ala His Cys Asn Ile Ser
 181    GCA CAT TGT AAC ATT AGT

        ProAspPro Linker
            BamHI
            ~~~~~~~~
        Pro Asp Pro
 199    CCG GAT CCA

        OR (Gly4Ser)3 Linker                                    BamHI
                                                                ~~~~~~~~
   Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
199GGT GGC GGT GGC TCA GGA GGC GGT GGA TCT GGC GGT GGA GGT TCG GAT CCA

        CD154 SHORT extracellular domain
208PDP  Glu Asn Ser Phe Glu Met Gln
250GS   GAA AAC AGC TTT GAA ATG CAA
229PDP  Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu
271GS   AAA GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG
274PDP  Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
316GS   GCC AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA
319PDP  Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys
361GS   TAC TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA
364PDP  Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
406GS   CAG CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA
409PDP  Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe
451GS   GTC ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT
454PDP  Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
496GS   ATA GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC
499PDP  Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly
541GS   TTA CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG
544PDP  Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
586GS   CAA CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT
589PDP  Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His
631GS   GCT TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT
634GS   Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** ***
676GS   GGC ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA
        XbaI
        ~~~~~~~
679PDP  Ser Arg
721GS   TCT AGA
```

Figure 3A.

Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

```
      HindIII
      ~~~~~~~~          Signal Peptide
                              Met  Leu  Tyr  Thr  Ser  Gln  Leu  Leu  Gly  Leu  Leu
    1 AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                    BglII
                                    ~~~~~~~~      HIV gp120 domain
      Leu  Phe  Trp  Ile  Ser  Ala  Ser  Arg  Ser  Met  Leu  Leu  Gly  Ile  Leu
   46 CTG TTT TGG ATC TCG GCT TCG AGA TCT ATG CTC CTT GGG ATA TTG
      Met  Ile  Cys  Ser  Ala  Thr  Glu  Lys  Leu  Trp  Val  Thr  Val  Tyr  Tyr
   91 ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT
      Gly  Val  Pro  Val  Trp  Arg  Glu  Ala  Thr  Thr  Thr  Leu  Phe  Cys  Ala
  136 GGG GTA CCT GTG TGG AGA GAA GCA ACC ACC ACT CTA TTT TGT GCA
      Ser  Asp  Ala  Lys  Ala  Tyr  Asp  Thr  Glu  Val  His  Asn  Val  Trp  Ala
  181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC
      Thr  His  Ala  Cys  Val  Pro  Thr  Asp  Pro  Asn  Pro  Gln  Glu  Val  Val
  226 ACA CAT GCC TGT GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA
      Leu  Gly  Asn  Val  Thr  Glu  Asn  Phe  Asn  Met  Trp  Lys  Asn  Asn  Met
  271 TTG GGA AAT GTG ACA GAA AAT TTT AAC ATG TGG AAA AAT AAC ATG
      Val  Asp  Gln  Met  His  Glu  Asp  Ile  Ile  Ser  Leu  Trp  Asp  Glu  Ser
  316 GTA GAT CAG ATG CAT GAG GAT ATA ATC AGT TTA TGG GAT GAA AGC
      Leu  Lys  Pro  Cys  Val  Lys  Leu  Thr  Pro  Leu  Cys  Val  Thr  Leu  Asn
  361 CTA AAG CCA TGT GTA AAA TTA ACC CCA CTC TGT GTT ACT TTA AAT
      Cys  Thr  Asn  Leu  Asn  Ile  Thr  Lys  Asn  Thr  Thr  Asn  Pro  Thr  Ser
  406 TGC ACT AAT TTG AAT ATC ACT AAG AAT ACT ACT AAT CCC ACT AGT
      Ser  Ser  Trp  Gly  Met  Met  Glu  Lys  Gly  Glu  Ile  Lys  Asn  Cys  Ser
  451 AGC AGC TGG GGA ATG ATG GAG AAA GGA GAA ATA AAA AAT TGC TCT
      Phe  Tyr  Ile  Thr  Thr  Ser  Ile  Arg  Asn  Lys  Val  Lys  Lys  Glu  Tyr
  496 TTC TAT ATC ACC ACA AGC ATA AGA AAT AAG GTA AAG AAA GAA TAT
      Ala  Leu  Phe  Asn  Arg  Leu  Asp  Val  Val  Pro  Ile  Glu  Asn  Thr  Asn
  541 GCA CTT TTT AAT AGA CTT GAT GTA GTA CCA ATA GAA AAT ACT AAT
      Asn  Thr  Lys  Tyr  Arg  Leu  Ile  Ser  Cys  Asn  Thr  Ser  Val  Ile  Thr
  586 AAT ACT AAG TAT AGG TTA ATA AGT TGT AAC ACC TCA GTC ATT ACA
      Gln  Ala  Cys  Pro  Lys  Val  Ser  Phe  Gln  Pro  Ile  Pro  Ile  His  Tyr
  631 CAG GCC TGT CCA AAG GTA TCC TTT CAG CCA ATT CCC ATA CAT TAT
      Cys  Val  Pro  Ala  Gly  Phe  Ala  Met  Leu  Lys  Cys  Asn  Asn  Lys  Thr
  676 TGT GTC CCG GCT GGG TTT GCG ATG CTA AAG TGT AAC AAT AAG ACA
      Phe  Asn  Gly  Ser  Gly  Pro  Cys  Thr  Asn  Val  Ser  Thr  Val  Gln  Cys
  721 TTC AAT GGA TCA GGA CCA TGC ACA AAT GTC AGC ACA GTA CAA TGT
      Thr  His  Gly  Ile  Arg  Pro  Val  Val  Ser  Thr  Gln  Leu  Leu  Leu  Asn
  766 ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT
      Gly  Ser  Leu  Ala  Glu  Glu  Asp  Ile  Val  Ile  Arg  Ser  Glu  Asn  Phe
  811 GGC AGT CTA GCA GAA GAA GAC ATA GTA ATT AGA TCT GAA AAT TTC
      Thr  Asp  Asn  Ala  Lys  Thr  Ile  Ile  Val  Gln  Leu  Asn  Glu  Ser  Val
  856 ACA GAC AAT GCT AAA ACC ATA ATA GTA CAG CTA AAT GAA TCT GTA
      Val  Ile  Asn  Cys  Thr  Arg  Pro  Asn  Asn  Asn  Thr  Arg  Arg  Arg  Leu
  901 GTA ATT AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA
      Ser  Ile  Gly  Pro  Gly  Arg  Ala  Phe  Tyr  Ala  Arg  Arg  Asn  Ile  Ile
  946 TCT ATA GGA CCA GGG AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA
      Gly  Asp  Ile  Arg  Gln  Ala  His  Cys  Asn  Ile  Ser  Arg  Ala  Lys  Trp
  991 GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG
      Asn  Asn  Thr  Leu  Gln  Gln  Ile  Val  Ile  Lys  Leu  Arg  Glu  Lys  Phe
 1036 AAT AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAA AAA TTT
      Arg  Asn  Lys  Thr  Ile  Ala  Phe  Asn  Gln  Ser  Ser  Gly  Gly  Asp  Pro
 1081 AGG AAT AAA ACA ATA GCC TTT AAT CAA TCC TCA GGA GGG GAC CCA
      Glu  Ile  Val  Met  His  Ser  Phe  Asn  Cys  Gly  Gly  Glu  Phe  Phe  Tyr
 1126 GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTC TTC TAC
      Cys  Asn  Thr  Ala  Gln  Leu  Phe  Asn  Ser  Thr  Trp  Asn  Val  Thr  Gly
 1171 TGT AAT ACA GCA CAA CTG TTT AAT AGT ACT TGG AAT GTT ACT GGA
      Gly  Thr  Asn  Gly  Thr  Glu  Gly  Asn  Asp  Ile  Ile  Thr  Leu  Gln  Cys
```

Figure 3A (continued).
Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

```
1216   GGG ACA AAT GGC ACT GAA GGA AAT GAC ATA ATC ACA CTC CAA TGC
       Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
1261   AGA ATA AAA CAA ATT ATA AAT ATG TGG CAG AAA GTA GGA AAA GCA
       Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn
1306   ATG TAT GCC CCT CCC ATC ACA GGA CAA ATT AGA TGT TCA TCA AAT
       Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu
1351   ATT ACA GGG CTG CTA CTA ACA AGA GAT GGA GGT AAT AGT ACT GAG
       Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp
1396   ACT GAG ACT GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC
       Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu
1441   AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AGA ATT GAA
       Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln
1486   CCA ATA GGA GTA GCA CCC ACC AGG GCA AAG AGA AGA ACA GTG CAA
       Arg Glu Lys Arg
1531   AGA GAA AAA AGA
```

(Gly$_4$Ser)$_3$ linker BamHI

```
                                                          ~~~~~~~~
       Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
1543   GGG GGA GGC GGT TCA GGA GGT GGA GGT TCT GGA GGT GGC GGA TCG GAT CCA
```

OR ProAspPro linker

```
         BamHI
         ~~~~~~~~
1543   Pro Asp Pro
       CCG GAT CCA
```

CD154 LONG FORM Extracellular Domain

```
1594GS      Arg Arg Leu Asp Lys Ile Glu Asp Glu
1552PDP     AGA AGG TTG GAC AAG ATA GAA GAT GAA
1621GS      Arg Asn Leu His Glu Asp Phe Val Phe Met Lys Thr Ile Gln Arg
1579PDP     AGG AAT CTT CAT GAA GAT TTT GTA TTC ATG AAA ACG ATA CAG AGA
1666GS      Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys Glu Glu
1624PDP     TGC AAC ACA GGA GAA AGA TCC TTA TCC TTA CTG AAC TGT GAG GAG
1711GS      Ile Lys Ser Gln Phe Glu Gly Phe Val Lys Asp Ile Met Leu Asn
1669PDP     ATT AAA AGC CAG TTT GAA GGC TTT GTG AAG GAT ATA ATG TTA AAC
1756GS      Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln Lys Gly
1714PDP     AAA GAG GAG ACG AAG AAA GAA AAC AGC TTT GAA ATG CAA AAA GGT
1801GS      Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala Ser
1759PDP     GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG GCC AGC
1846GS      Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr Tyr
1804PDP     AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA TAC TAC
1891GS      Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln Leu
1849PDP     ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA CAG CTG
1936GS      Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
1894PDP     ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA GTC ACC
1981GS      Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala
1939PDP     TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT ATA GCC
2026GS      Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
1984PDP     AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC TTA CTC
2071GS      Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln
2029PDP     AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG CAA CAA
2116GS      Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
2074PDP     TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT GCT TCG
2161GS      Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr
2119PDP     GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT GGC ACT
                                                        XbaI
2206GS      Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** *** Ser Arg
2164PDP     GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA TCT AGA
```

Figure 3B.

Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

```
       HindIII
       ~~~~~~~~         Signal Peptide
                        Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
   1   AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                    BglII
                                    ~~~~~~~~    HIV gp120 domain
       Leu Phe Trp Ile Ser Ala Ser Arg Ser Met Leu Leu Gly Ile Leu
  46   CTG TTT TGG ATC TCG GCT TCG AGA TCT ATG CTC CTT GGG ATA TTG
       Met Ile Cys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr
  91   ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT
       Gly Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala
 136   GGG GTA CCT GTG TGG AGA GAA GCA ACC ACC ACT CTA TTT TGT GCA
       Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala
 181   TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC
       Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val
 226   ACA CAT GCC TGT GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA
       Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met
 271   TTG GGA AAT GTG ACA GAA AAT TTT AAC ATG TGG AAA AAT AAC ATG
       Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser
 316   GTA GAT CAG ATG CAT GAG GAT ATA ATC AGT TTA TGG GAT GAA AGC
       Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn
 361   CTA AAG CCA TGT GTA AAA TTA ACC CCA CTC TGT GTT ACT TTA AAT
       Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro Thr Ser
 406   TGC ACT AAT TTG AAT ATC ACT AAG AAT ACT ACT AAT CCC ACT AGT
       Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser
 451   AGC AGC TGG GGA ATG ATG GAG AAA GGA GAA ATA AAA AAT TGC TCT
       Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
 496   TTC TAT ATC ACC ACA AGC ATA AGA AAT AAG GTA AAG AAA GAA TAT
       Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn
 541   GCA CTT TTT AAT AGA CTT GAT GTA GTA CCA ATA GAA AAT ACT AAT
       Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr
 586   AAT ACT AAG TAT AGG TTA ATA AGT TGT AAC ACC TCA GTC ATT ACA
       Gln Ala Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr
 631   CAG GCC TGT CCA AAG GTA TCC TTT CAG CCA ATT CCC ATA CAT TAT
       Cys Val Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr
 676   TGT GTC CCG GCT GGG TTT GCG ATG CTA AAG TGT AAC AAT AAG ACA
       Phe Asn Gly Ser Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys
 721   TTC AAT GGA TCA GGA CCA TGC ACA AAT GTC AGC ACA GTA CAA TGT
       Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
 766   ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT
       Gly Ser Leu Ala Glu Glu Asp Ile Val Ile Arg Ser Glu Asn Phe
 811   GGC AGT CTA GCA GAA GAA GAC ATA GTA ATT AGA TCT GAA AAT TTC
       Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Glu Ser Val
 856   ACA GAC AAT GCT AAA ACC ATA ATA GTA CAG CTA AAT GAA TCT GTA
       Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu
 901   GTA ATT AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA
       Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile
 946   TCT ATA GGA CCA GGG AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA
       Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp
 991   GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG
       Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe
1036   AAT AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAA AAA TTT
       Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp Pro
1081   AGG AAT AAA ACA ATA GCC TTT AAT CAA TCC TCA GGA GGG GAC CCA
       Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
1126   GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTC TTC TAC
       Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly
1171   TGT AAT ACA GCA CAA CTG TTT AAT AGT ACT TGG AAT GTT ACT GGA
```

Figure 3B (Continued).
Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

```
        Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
1216    GGG ACA AAT GGC ACT GAA GGA AAT GAC ATA ATC ACA CTC CAA TGC
        Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
1261    AGA ATA AAA CAA ATT ATA AAT ATG TGG CAG AAA GTA GGA AAA GCA
        Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn
1306    ATG TAT GCC CCT CCC ATC ACA GGA CAA ATT AGA TGT TCA TCA AAT
        Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu
1351    ATT ACA GGG CTG CTA CTA ACA AGA GAT GGA GGT AAT AGT ACT GAG
                    BglII
                    ~~~~~~~~
        Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp
1396    ACT GAG ACT GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC
        Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu
1441    AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AGA ATT GAA
        Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln
1486    CCA ATA GGA GTA GCA CCC ACC AGG GCA AAG AGA AGA ACA GTG CAA
        Arg Glu Lys Arg
1531    AGA GAA AAA AGA

        (Gly4Ser)3 linker                                    BamHI

        Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
1543    GGG GGA GGC GGT TCA GGA GGT GGA GGT TCT GGA GGT GGC GGA TCG GAT CCA

        OR ProAspPro linker
          BamHI

        Pro Asp Pro
1543    CCG GAT CCA

        CD154 SHORT FORM Extracellular Domain
1594GS      Glu Asn Ser Phe Glu Met Gln Lys
1552PDP     GAA AAC AGC TTT GAA ATG CAA AAA
1618GS      Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala
1576PDP     GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG GCC
1663GS      Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr
1621PDP     AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA TAC
1708GS      Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
1666PDP     TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA CAG
1753GS      Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val
1711PDP     CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA GTC
1798GS      Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
1756PDP     ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT ATA
1843GS      Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu
1801PDP     GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC TTA
1888GS      Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
1846PDP     CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG CAA
1933GS      Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala
1891PDP     CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT GCT
1978GS      Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
1936PDP     TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT GGC
                                                            XbaI
                                                            ~~~
2023GS      Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** *** Ser
1981PDP     ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA TCT
            XbaI
            ~~~
2068GS      Arg
2026PDP     AGA
```

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	
First Named Inventor	Jeffrey Ledbetter
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DNA Vaccines Encoding Antigen Linked to a Domain That Binds CD40.

(Title of the Invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) [   ] as United States Application Number or PCT International Application Number [   ] and was amended on (MM/DD/YYYY) [   ] (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
US60/159,690	10/14/99	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
☐ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] *OR* ☒ Correspondence address below

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Address					
City	Shoreline	State	WA	ZIP	98177-3227
Country	USA	Telephone	(206) 546-0473	Fax	(206) 546-6002

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Jeffrey A.	Ledbetter

Inventor's Signature	Jeffrey A Ledbetter 10/13/00					Date	10/13/00
Residence: City	Shoreline	State	WA	Country	USA	Citizenship	USA
Post Office Address	18798 Ridgefield Road N.W.						
Post Office Address							
City	Shoreline	State	WA	ZIP	98177-3227	Country	USA

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Martha			Hayden-Ledbetter					
Inventor's Signature	Martha Hayden-Ledbetter					**Date**	10/13/00 10/13/00	
Residence: City	Shoreline	**State**	WA	**Country**	USA	**Citizenship**	USA	
Post Office Address	18798 Ridgefield Road N.W.							
Post Office Address								
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Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Inventor's Signature						**Date**		
Residence: City		**State**		**Country**		**Citizenship**		
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Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
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SEQUENCE LISTING

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tcggatccaa gaaggttgga caagatagaa gatgaaagga atcttcatga agattttgta 300

ttcatgaaaa cgatacagag atgcaacaca ggagaaagat ccttatcctt actgaactgt 360

gaggagatta aaagccagtt tgaaggcttt gtgaaggata taatgttaaa caaagaggag 420

acgaagaaag aaaacagctt tgaaatgcaa aaaggtgatc agaatcctca aattgcggca 480

catgtcataa gtgaggccag cagtaaaaca acatctgtgt tacagtgggc tgaaaaagga 540

tactacacca tgagcaacaa cttggtaacc ctggaaaatg ggaaacagct gaccgttaaa 600

agacaaggac tctattatat ctatgcccaa gtcaccttct gttccaatcg ggaagcttcg 660

agtcaagctc catttatagc cagcctctgc ctaaagtccc ccggtagatt cgagagaatc 720

ttactcagag ctgcaaatac ccacagttcc gccaaacctt gcgggcaaca atccattcac 780

ttgggaggag tatttgaatt gcaaccaggt gcttcggtgt ttgtcaatgt gactgatcca 840

agccaagtga gccatggcac tggcttcacg tcctttggct tactcaaact cgagtgataa 900

tctaga 906

<210> 17
<211> 865
<212> DNA
<213> HIV-HUMAN FUSION CDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop + ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(865)
<223> CD154 extracellular domain
long form from amino acids 48-261+Glu
binds CD40

<400> 17
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtcc ggatccaaga aggttggaca agatagaaga tgaaaggaat 240

cttcatgaag attttgtatt catgaaaacg atacagagat gcaacacagg agaaagatcc 300

ttatccttac tgaactgtga ggagattaaa agccagtttg aaggctttgt gaaggatata 360

atgttaaaca aagaggagac gaagaaagaa aacagctttg aaatgcaaaa aggtgatcag 420

aatcctcaaa ttgcggcaca tgtcataagt gaggccagca gtaaaacaac atctgtgtta 480

cagtgggctg aaaaaggata ctacaccatg agcaacaact tggtaaccct ggaaaatggg 540

aaacagctga ccgttaaaag acaaggactc tattatatct atgcccaagt caccttctgt 600

tccaatcggg aagcttcgag tcaagctcca tttatagcca gcctctgcct aaagtccccc 660

ggtagattcg agagaatctt actcagagct gcaaataccc acagttccgc caaaccttgc 720

gggcaacaat ccattcactt gggaggagta tttgaattgc aaccaggtgc ttcggtgttt 780

gtcaatgtga ctgatccaag ccaagtgagc catggcactg gcttcacgtc ctttggctta 840

ctcaaactcg agtgataatc tagat 865

<210> 18
<211> 726
<212> DNA
<213> HIV-HUMAN FUSION CDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop plus ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(726)
<223> CD154 extracellular domain
short form from amino acids 108-261+Glu
binds CD40

<400> 18
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtgg tggcggtggc tcaggaggcg gtggatctgg cggtggaggt 240

tcggatccag aaaacagctt tgaaatgcaa aaaggtgatc agaatcctca aattgcggca 300

catgtcataa gtgaggccag cagtaaaaca acatctgtgt tacagtgggc tgaaaaagga 360

tactacacca tgagcaacaa cttggtaacc ctggaaaatg ggaaacagct gaccgttaaa 420

agacaaggac tctattatat ctatgcccaa gtcaccttct gttccaatcg ggaagcttcg 480

agtcaagctc catttatagc cagcctctgc ctaaagtccc ccggtagatt cgagagaatc 540

ttactcagag ctgcaaatac ccacagttcc gccaaacctt gcgggcaaca atccattcac 600

ttgggaggag tatttgaatt gcaaccaggt gcttcggtgt ttgtcaatgt gactgatcca 660

agccaagtga gccatggcac tggcttcacg tcctttggct tactcaaact cgagtgataa 720

tctaga 726

<210> 19
<211> 684
<212> DNA
<213> HIV-human fusion cDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> Synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop with ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(684)
<223> human CD154 extracellular domain
short form from amino acids 108-261+Glu
binds to CD40

<400> 19
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtcc ggatccagaa aacagctttg aaatgcaaaa aggtgatcag 240

aatcctcaaa ttgcggcaca tgtcataagt gaggccagca gtaaaacaac atctgtgtta 300

cagtgggctg aaaaaggata ctacaccatg agcaacaact tggtaaccct ggaaaatggg 360

aaacagctga ccgttaaaag acaaggactc tattatatct atgcccaagt caccttctgt 420

tccaatcggg aagcttcgag tcaagctcca tttatagcca gcctctgcct aaagtccccc 480

ggtagattcg agagaatctt actcagagct gcaaataccc acagttccgc caaaccttgc 540

gggcaacaat ccattcactt gggaggagta tttgaattgc aaccaggtgc ttcggtgttt 600

gtcaatgtga ctgatccaag ccaagtgagc catggcactg gcttcacgtc ctttggctta 660

ctcaaactcg agtgataatc taga 684

<210> 20
<211> 742
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> synthetic secretory signal peptide

<220>

<221> DOMAIN
<222> (21)..(526)
<223> HIV gp120 domain with (gly4ser)3 linker

<220>
<221> BINDING
<222> (529)..(742)
<223> CD154 extracellular domain
long form from amino acids 48 (Arg) to 261 (Leu)+Glu

<400> 20

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                  10                  15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
            20                  25                  30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
        35                  40                  45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
    50                  55                  60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65                  70                  75                  80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
                85                  90                  95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
            100                 105                 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
        115                 120                 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
    130                 135                 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145                 150                 155                 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
                165                 170                 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
            180                 185                 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
        195                 200                 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
    210                 215                 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225                 230                 235                 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
                245                 250                 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
            260                 265                 270
```

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
        275   280   285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
    290   295   300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305   310   315   320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
                325   330   335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
            340   345   350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
        355   360   365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
    370   375   380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385   390   395   400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
                405   410   415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
            420   425   430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
        435   440   445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
    450   455   460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465   470   475   480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
                485   490   495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Gly Gly
            500   505   510

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg
        515   520   525

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
    530   535   540

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
545   550   555   560

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
                565   570   575

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
            580   585   590

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
        595   600   605

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
    610   615   620

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
625 630 635 640

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
645 650 655

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
660 665 670

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
675 680 685

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
690 695 700

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
705 710 715 720

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
725 730 735

Gly Leu Leu Lys Leu Glu
740

<210> 21
<211> 728
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(513)
<223> HIV gp120 domain plus ProAspPro linker

<220>
<221> BINDING
<222> (514)..(728)
<223> CD154 extracellular domain
long form from amino acids 48 (Arg) to 261 (Leu)+Glu
Binds CD40

<400> 21

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
20 25 30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
35 40 45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
50 55 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro

65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

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Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
            420                 425                 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
        435                 440                 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
    450                 455                 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465                 470                 475                 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
                485                 490                 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Pro Asp
            500                 505                 510

Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
        515                 520                 525

Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
    530                 535                 540

Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
545                 550                 555                 560

Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
                565                 570                 575

Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
            580                 585                 590

Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
        595                 600                 605

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
    610                 615                 620

Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
625                 630                 635                 640

Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
                645                 650                 655

Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
            660                 665                 670

Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
        675                 680                 685

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
    690                 695                 700

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
705                 710                 715                 720

Ser Phe Gly Leu Leu Lys Leu Glu
                725
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<210> 22
<211> 682
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(525)
<223> HIV gp120 domain plus (gly4ser)3 linker

<220>
<221> DOMAIN
<222> (528)..(682)
<223> CD154 extracellular domain
short form from amino acids 108 (Glu) to 261 (Leu)+Glu
Binds CD40

<400> 22

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                  10                  15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
            20                  25                  30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
        35                  40                  45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
    50                  55                  60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65                  70                  75                  80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
                85                  90                  95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
            100                 105                 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
        115                 120                 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
    130                 135                 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145                 150                 155                 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
                165                 170                 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
            180                 185                 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
        195                 200                 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
    210                 215                 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
```

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225                 230                 235                 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
                245                 250                 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
            260                 265                 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
        275                 280                 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
    290                 295                 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305                 310                 315                 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
                325                 330                 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
            340                 345                 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
        355                 360                 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
    370                 375                 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385                 390                 395                 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
                405                 410                 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
            420                 425                 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
        435                 440                 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
    450                 455                 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465                 470                 475                 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
                485                 490                 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Gly Gly
            500                 505                 510

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Glu
        515                 520                 525

Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala
    530                 535                 540

His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp
545                 550                 555                 560

Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu
                565                 570                 575
```

Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr
580 585 590

Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro
595 600 605

Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
610 615 620

Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
625 630 635 640

Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
645 650 655

Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
660 665 670

Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
675 680

<210> 23
<211> 668
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(513)
<223> HIV gp120 domain with ProAspPro linker

<220>
<221> BINDING
<222> (514)..(668)
<223> CD154 extracellular domain
short form from amino acids 108 (Glu) to 261 (Leu)+Glu
Binds to CD40

<400> 23

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
20 25 30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
35 40 45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
50 55 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
420 425 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr

```
        435                 440                 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
    450                 455                 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465                 470                 475                 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
                485                 490                 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Pro Asp
            500                 505                 510

Pro Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile
        515                 520                 525

Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu
    530                 535                 540

Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr
545                 550                 555                 560

Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr
                565                 570                 575

Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln
            580                 585                 590

Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu
        595                 600                 605

Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys
    610                 615                 620

Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
625                 630                 635                 640

Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
                645                 650                 655

Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
            660                 665
```

<210> 24
<211> 294
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(77)
<223> HIV gp120 V3 loop plus (gly4ser)3 linker

<220>
<221> BINDING
<222> (80)..(294)
<223> CD154 extracellular domain

long form from amino acids 48 (Arg) to 261 (Leu)+Glu
binds CD40

<400> 24

```
Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                  10                  15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            20                  25                  30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
        35                  40                  45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly
    50                  55                  60

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg
65                  70                  75                  80

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
                85                  90                  95

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
            100                 105                 110

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
        115                 120                 125

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
    130                 135                 140

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
145                 150                 155                 160

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
                165                 170                 175

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
            180                 185                 190

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
        195                 200                 205

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
    210                 215                 220

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
225                 230                 235                 240

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
                245                 250                 255

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
            260                 265                 270

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
        275                 280                 285

Gly Leu Leu Lys Leu Glu
    290
```

<210> 25
<211> 280

<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(65)
<223> HIV gp120 V3 loop plus ProAspPro linker

<220>
<221> BINDING
<222> (66)..(280)
<223> CD154 extracellular domain
long form from amino acids 48 (Arg) to 261 (Leu)+Glu
binds CD40

<400> 25

```
Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                  10                  15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            20                  25                  30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
        35                  40                  45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
    50                  55                  60

Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
65                  70                  75                  80

Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
                85                  90                  95

Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
            100                 105                 110

Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
        115                 120                 125

Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
    130                 135                 140

Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
145                 150                 155                 160

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
                165                 170                 175

Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
            180                 185                 190

Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
        195                 200                 205

Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
```

```
    210                 215                 220

Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
225                 230                 235                 240

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
                245                 250                 255

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
            260                 265                 270

Ser Phe Gly Leu Leu Lys Leu Glu
        275                 280
```

<210> 26
<211> 234
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(77)
<223> HIV gp120 V3 loop plus (gly4ser)3 linker

<220>
<221> BINDING
<222> (80)..(234)
<223> CD154 extracellular domain
short form from amino acids 108 (Glu) to 261 (Leu)+Glu
binds CD40

<400> 26

```
Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                   10                  15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            20                  25                  30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
        35                  40                  45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly
    50                  55                  60

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Glu
65                  70                  75                  80

Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala
                85                  90                  95

His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp
            100                 105                 110

Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu
        115                 120                 125
```

```
Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr
    130                 135                 140

Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro
145                 150                 155                 160

Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
                165                 170                 175

Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
            180                 185                 190

Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
        195                 200                 205

Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
    210                 215                 220

Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
225                 230

<210>  27
<211>  220
<212>  PRT
<213>  HIV-HUMAN FUSION PROTEIN

<220>
<221>  SIGNAL
<222>  (1)..(20)
<223>  synthetic secretory signal peptide


<220>
<221>  DOMAIN
<222>  (21)..(65)
<223>  HIV gp120 V3 loop plus ProAspPro linker


<220>
<221>  BINDING
<222>  (66)..(220)
<223>  CD154 extracellular domain from amino acids 108 (Glu)-261(Leu)+Gl
       u
       Binds CD40


<400>  27

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                   10                  15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            20                  25                  30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
        35                  40                  45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
    50                  55                  60

Pro Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile
65                  70                  75                  80

Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu
                85                  90                  95
```

```
Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr
               100                 105                 110

Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr
           115                 120                 125

Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln
       130                 135                 140

Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu
145                 150                 155                 160

Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys
                    165                 170                 175

Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
               180                 185                 190

Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
           195                 200                 205

Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
       210                 215                 220
```